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SPECTROPHOTOMETRIC ANALYSIS OF BOVINE SERUM ALBUMIN IN PRESENCE OF 1-(4-AMINOPHENYL)-3-PHENYLPROP-2-EN-1-ONES

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ABSTRACT: Proteins, which are soluble under normal physiological conditions, sometimes form insoluble aggregates with serious medical implications. Bovine serum albumin (BSA), a structural analogue of human serum albumin. Bovine Serum Albumin (BSA) has been used as a carrier, and stabilizing agent, for insoluble fatty acids. The chemistry of chalcone has been recognized as a significant field of study. An interesting feature of chalcones is that they serve as starting materials for the synthesis of various heterocyclic compounds such as pyrimidines, pyrazolines, flavones, flavonols, flavanones, aurones and benzoylcoumarones as well as certain compounds like deoxybenzoins and hydantions which are of some therapeutic importance. A series of chalcones was synthesized by the Claisen-Schmidt condensation and the structures of 1-(4-aminophenyl)-3-phenylprop-2-en-1-ones were established with the help of IR and NMR study, then their effect was observed on bovine serum albumin. We have found that the synthesized chalcones interacted with bovine serum albumin and produce a great effect on their presence.

INTRODUCTION: Serum albumins are one of the most abundant proteins in blood plasma, which are the major soluble protein constituents of the circulatory system. They play a dominant role in the transport and deposition of endogenous and exogenous ligands in blood, since serum albumins often increase the apparent solubility of hydrophobic drugs in plasma and modulate their delivery to cells in vivo and in vitro. Hence, it is important and necessary to study the interaction of drug with BSA at molecular level [1]. Investigating the interaction of drugs to serum albumins can elucidate the properties of drug-protein complex, as it may provide useful information of the structural features that determine the therapeutic effectiveness of drugs.

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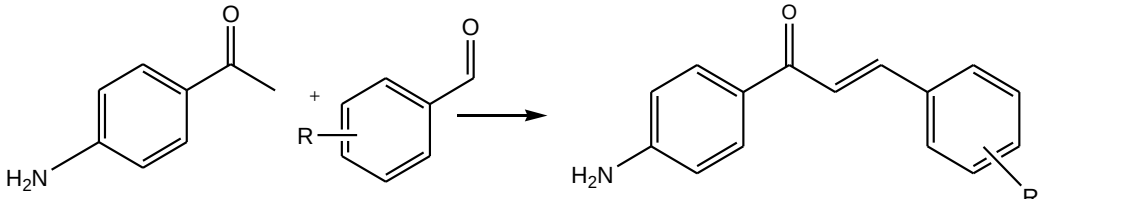
Interaction with albumin could also be critical for understanding the drug toxicity and its distribution in the organism. It has been an interesting research field in life science, chemistry and clinical medicine.

Chalcones (1, 3-diaryl-2-propen-1-ones and the derivatives of this basic structure belong to the flavonoid family, and commonly possess interesting biological activities such as anticancer, antimicrobial, antiprotozoal, antihistaminic, anti-inflammatory and many other activities, as reviewed elsewhere [2, 3]. Currently, several of these derivatives have been approved for clinical use or are already being tested in humans[4]. Beyond these attractive clinical properties, new bio- logical activities of chalcones such as in antiblood platelets coagulation, antihemostasis and microbial resistances are currently under investigation[5]. Proteins are one of the preferential targets for biologically active chalcones apart from their origin [5] (natural or synthetic).

We have reported the interaction of some series of chalcones with BSA. In continuation of our previous work, with 1-(5'-chloro-2'-hydroxyphenyl)-3-(4"-substituted phenyl)-prop-2-en-1-one and their methoxy derivatives [6], 1-phenyl-3-(substituted phenyl)-prop-2-en-1-one [7], 1-(2'-furyl)-3-(substituted phenyl)-prop-2-en-1-one [8],1-(2'-thienyl)-3-(substituted phenyl)-prop-2-en-1-one [9],1-(4-hydroxyphenyl)-3 (substituted phenyl)-2-propen-1-ones and 1-(4-nitrophenyl)-3-(substituted phenyl)-2-propen-1-ones [10], 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones [11], bischalcones [12],1-(4-methylphenyl)-3-phenylprop-2-en-1-ones [13], 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones [14], 1-(4-(benzylideneamino)phenyl)-3-phenylprop-2-en-1-ones[15] and 1-(naphthalen-3-yl)-3-phenylprop-2-en-1-ones[16] with bovine serum albumin, we here report the interaction of bovine serum albumin with 1-(4-aminophenyl)-3-phenylprop-2-en-1-ones. This protein is involved in the transportation of a number of compounds including drugs. It is also reported that there is about 80% primary sequence identity between bovine serum albumin and human serum albumin [17]. It is also suggested that the present study performedwith BSA can give an insight about the interaction of chalcones with human serum albumin.

MATERIALS AND METHODS: The reaction progress and purity of products were monitored by thin layer chromatography. Thin layer chromatography was performed with silica-gel G (suspended in $CHCI_3$-EtOH) and plates were viewed under Iodine vapors. Melting points were determined by electrochemical capillary Melting points apparatus and are uncorrected. Elisa plate reader, Systronic make was used for measuring absorbance in the visible range. The Lab-India made Spectrofuge (model 16M) was used for centrifugation purpose.

Synthesis of Chalcones- A series of chalcones 1-(4-aminophenyl)-3-phenylprop-2-en-1-one was synthesized by the grinding of substituted benzaldehyde (0.01 mole) with 4-aminoaceto-phenone (0.01 mole) in presence of potassium hydroxide (0.01 mole) respectively with a mortar and pestle. The progress of reaction and the purity of the products were confirmed through TLC. The structures were confirmed by their IR and ^{1}HNMR spectra.



4-aminoacetophenone subsitutedbenzaldehyde 1-(4-aminophenyl)-3-subsitutedphenylprop-2-en-1-one

Reaction of chalcones with Bovine Serum Albumin- To 10 ml solution of 0.1mM BSA, 1ml solution of 50 mM chalcone solution was added drop wise with constant stirring. After interaction between chalcone and BSA, some albumin gets precipitated. The remaining protein in solution was estimated by biuret method [18]. The results are presented in **Figure 1.**

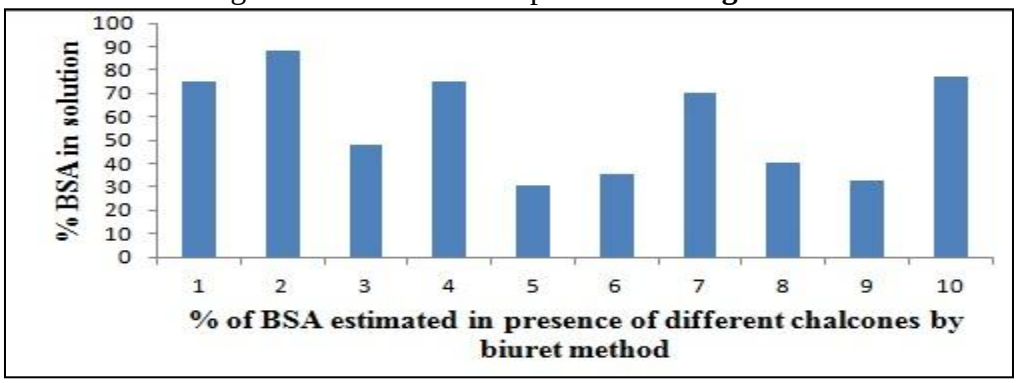


FIGURE 1: THE RESULTS PRESENTED ARE CALCULATED AS % OF BSA LEFT IN SOLUTION AFTER INTERACTION WITH CHALCONE WITH RESPECT TO CONTROL WHERE NO CHALCONE WAS ADDED BUT AN EQUAL AMOUNT OF SOLVENT WAS ADDED

EXPERIMENTAL: A series 1-(4-aminophenyl)-3-phenylprop-2-en-1-one was synthesized in good yields by Claisen Schmidt reaction between substituted benzaldehydes and 4-aminoacetophenone. Their IR and ^{1}HNMR data are reported in **Table 1 & 2.**

TABLE 1: IR DATA [Y MAX (CM^{-1})] OF CHALCONES ($H_2NC_6H_4$-CO-CH=CH-C_6H_4R)

Comp. No.	R	C=O]	[C=C]	[CH]	[O-N-Osym]	[O-N-O asym]
1	*H*	1654	1597	3078	-	-
2	*o*-Cl	1657	1597	3091	-	-
3	*m*-Cl	1658	1599	2977	-	-
4	*p*-Cl	1657	1591	2921	-	-
5	*o*-OMe	1654	1591	3142	-	-
6	*m*-OMe	1655	1605	2873	-	-
7	*p*-OMe	1655	1603	2877	-	-
8	*o*-NO2	1655	1605	2876	1344	1525
9	*m*-NO2	1655	1605	2876	1344	1526
10	*p*-NO2	1655	1603	2812	1335	1529

TABLE 2: ^{1}HNMR (Δ PPM) DATA OBTAINED FOR CHALCONES ($H_2NC_6H_4$-CO-CH=CH-C_6H_4R)

Comp No	R	H-2	H-3	J2-3 (Hz) -	Ar-H	3H,-OCH3
1	H	6.923 d)	7.841(d)	15.8	7.132-8.142(m)	-
2	*o*-Cl	7.323 (d)	8.645(d)	15.2	7.187-8.405(m)	-
3	*m*-Cl	7.320 (d)	7.891(d)	15.5	7.122-8.516(m)	-
4	*p*-Cl	7.450 (d)	7.882 (d)	15.7	7.121-8.326(m)	-
5	*o*-OCH_3	7.439 (d)	7.841 (d)	15.8	7.151-8.406(m)	3.824
6	*m*-OCH_3	7.562 (d)	8.111 (d)	15.8	7.670-8.216(m)	3.932
7	p-OCH_3	7.534 (d)	8.076(d)	15.6	7.128-8.223(m)	3.861
8	*o*-NO_2	7.386(d)	7.585 (d)	15.6	7.189-8.312(m)	-
9	*m*-NO_2	7.397 (d)	7.685 (d)	15.3	7.199-8.343(m)	-
10	*p*-NO_2	6.671 (d)	7.546 (d)	15.3	7.156-8.456(m)	-

In **Table 3**, ^{1}HNMR ($CDCl_3$) data of different chalcones are presented. It was observed that C-2 and C-3 protons resonated as doublets with coupling constant ~ 15 Hz. The stereochemistry across C-2, C-3 double bond is Trans. The other protons were revealed at their respective position.

TABLE 3: EXPERIMENTAL ANALYSIS OF SYNTHESIZED CHALCONES ($H_2NC_6H_4$-CO-CH=CH-C_6H_4R)

Comp. No.	R-	% of BSA left in solution after interaction with chalcones
1.	H	74.92
2.	*o*-Cl	88.34
3.	*m*-Cl	47.83
4.	*p*-Cl	75.41
5.	*o*-OCH_3	30.76
6.	*m*-OCH_3	35.43
7.	*p*-OCH_3	70.56
8.	*o*-NO_2	40.71
9.	*m*-NO_2	32.98
10.	*p*-NO_2	77.3

RESULTS AND DISCUSSION: The most widely used method used for the synthesis of chalcones involves Claisen-Schmidt condensation of substituted arylaldehyde with the arylmethyl ketones with the help of mortar and pestle by solvent free synthesis. In the present work we report the synthesis of one series i.e. (4-aminophenyl)-3-phenylprop-2-en-1-one by the reaction of substituted benzaldehydes with 4-methylacetophenone and in the presence of a base. The synthesis of different chalcones was established by their spectral data. In the IR spectra

of chalcones (1-10) as mentioned in Table 1, the peak at 1651 – 1659 cm^{-1} represent >C=O stretching vibrations which indicate the presence of carbonyl group in conjugation with highly unsaturated system and the results suggests the presence of α, β – unsaturated carbonyl group in the synthesized compounds.

The synthesis of chalcones is characterized by the presence of two doublets around δ 7.4 - 6.7 and δ 8.1 - 7.4. These represents C-2 and C-3 protons and the geometry across the double bond has been found out to be trans as doublets with coupling constant $J_{2,3}$ is ~ 15.7 - 15.0 Hz. The aryl and other protons were revealed at their respective position. After establishing the structures of 1-(4-aminophenyl)-3-phenylprop-2-en-1-one their effect was observed on BSA in solution.

We have earlier reported spetrophotometric analysis of BSA in presence of different series of chalcones[6-16]. In the present work, the results are presented on the basis of interaction of serum protein with synthesized 1-(4-aminophenyl)-3-phenylprop-2-en-1-one **(Figure 1).**

The chalcones possess α, β-unsaturated ketone moiety and are therefore highly reactive. The moiety C_2-C_3 double bond is most nucleophilic group available and therefore has been used as a tool for the synthesis of large number of heterocycle compound [19].

In proteins also, a number of side chain groups such as thiol, amino, imidazole, alcohol etc. are available. Any of these nucleophilic groups can react with C2-C3 double bond of chalcones.

We propose that nucleophilic groups of BSA react with α, β-unsaturated group in an effective manner. The results suggest that 1-(4-aminophenyl)-3-(2-methoxyphenyl)-prop-2-en-1-one is most reactive chalcone as it decreased the availability of BSA in solution to maximum extent.

The resulting interactions may cause a change in the three dimensional structure of albumin under study and finally resulting its precipitation out of solution.

CONCLUSION: To conclude, we have synthesized a series i.e. 1-(4-aminophenyl)-3-phenylprop-2-en-1-one; by Claisen-Schmidt condensation successfully and has been characterized with the help of IR and ^1H NMR spectra. These α, β-unsaturated compounds may possess diverse pharmacological activities. It has been found that these chalcones interact with the bovine serum albumin, a protein mainly responsible for the transportation of a number of compounds.

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